Terpenoids in flax response to fusariose

ABSTRACT

Flax is a valued crop, which delivers such raw products like oil and fibre. Currently, thank to modern technologies, virtually all its parts can be utilized, including those that were thought to be by-products, that is shives or seedcakes, which are the main source of bio-active compounds. Despite that the conditions for flax cultivation are ideal in our country, its acreage decreases. It is connected with its susceptibility to diseases, among which fungi of *Fusarium* genus are most dangerous, including *F. oxysporum* is a flax-specific pathogen.

Depending on the pathogen type (specific/inspecific, necrotroph/biotroph), plants have developed various defense mechanisms, which involve different compounds and processes to stop the infection. According to the literature, PR proteins, phenylpropanoids or poliamines, are all involved in the response to pathogen attack, while there are only few reports on the role of terpenoids. Also, the mechanism of *Fusarium* infection in flax is not fully recognized.

The main aim of the study was the assessment of the selected terpenoid role in the response to infection with fungi of *Fusarium* genus. As the terpenoids are highly diverse group of secondary metabolites, particular analysis of three groups of these compounds: carotenoids, tocopherols and sterols was performed. Flax nucleotide sequences of selected genes involved in the synthesis of the studied compounds were identified. 41 terpenoid gene sequences were identified in this study of which 24 were placed in the NCBI database.

In flax seedlings treated with pathogen (in 5 time points: 6h, 12h, 24h, 36h, 48h after infection), expression levels of the identified genes were analyzed and metabolites were assayed. Moreover, the influence of pure compounds (analytical standards) on pathogen growth was investigated.

The obtained results indicate that in the case of *Fusarium* infection the non-mevalonate pathway is activated in flax, which leads to the synthesis of such compounds as carotenoids and tocopherols. Synthesis of phytosterols, deriving from the mevalonate pathway, slightly decreased, which was evidenced by measuring the levels of the sterols and expression of genes related to their biosynthesis. The content of tocopherols increased slightly during the infection, which relates to the growth in the expression level of the adequate genes. However, α-tocopherol did not show inhibitory effect on the pathogen growth. Increased synthesis of tocopherols can be thus connected with the necessity of quenching free radicals produced during the infection. The level of carotenoids in the course of the infection increased initially to drop down slightly below the control level during the following hours. The carotenoids, similarly to tocopherol, did no inhibit pathogen growth, though they influence its metabolism. Therefore, the level of expression of the genes connected with carotenoid oxidative cleavage and ABA synthesis. Expression of *nced3* and *nced6* genes, responsible for carotenoid oxidative cleavage to the precursors of abscisic acid, was significantly increased. Equally, the expression of genes directly connected with ABA

synthesis was higher. This was reflected in the content of ABA, which was increasing from the very beginning of the infection. The abscisic acid is considered a signal molecule, which triggers appropriate defense mechanisms in plants, similar to salicylic or jasmonic acids. About 10% of genes in plant genome possess *cis*-regulatory elements sensitive to ABA in their promoter sequences. One of such genes is callose synthase *pmr4*, which participation in the response to infection was confirmed in many cases. *Pmr4* gene sequence was identified in flax within this study. Its expression level, as well as callose content was determined. Both of these values increase during the infection.

The obtained results indicate that within the terpenoid biosynthesis pathway the route leading to abscisic acid synthesis plays a key role in the response to *Fusarium* infection. The increased content of ABA in cell triggers different resistance mechanisms, including synthesis of callose, which contributes to structure of papillae – a natural barrier to pathogen.

Additional aspect of the study was the investigation of how the manipulation of carotenoid synthesis influences the phenotype and resistance of flax.

The first plant type analyzed was flax with the repression of lycopen β-cyclase. The expression level of genes involved in terpenoid biosynthesis and adequate metabolite contents were measured in two selected lines (L9 and L18). The content of both carotenoids and tocopherols was decreased in these plants, while the content of ABA was slightly elevated. The seeds produced by those plants were more resistant to *Fusarium* infection, though the mechanism of this resistance as yet remains unknown. Also, an attempt to clarify the mechanism of *lcb* gene silencing was undertaken. For that purpose the methylation level in those plants as well as methylation pattern in the lycopen β-cyclase gene were analyzed.

The second type of investigated plants was flax with increased carotenoid synthesis. To obtain such plants bacterial genes from *Erwinia uredovora* coding for phytoene synthase (*crtB*) and carotene desaturase (*crtI*) were introduced to flax genome in the way of agrotransformation. The obtained plant transformants have increased carotenoid content in seeds.